PRODUCTION AND SEPARATION OF GLUCOSE FROM CELLULOSE HYDROLYSATES USING MEMBRANE REACTOR: EFFECT OF TRANSMEMBRANE PRESSURE AND CROSS FLOW VELOCITY

MOHD HAFIZUDDIN BIN ZAHARI

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

JANUARY 2012

ABSTRACT

Sawdust from hardwood contain large amount of cellulose and hemicellulose. Enzymatic hydrolysis of cellulose has larger potential in fulfil global food and energy demand by reducing sugar production such as glucose. In this study, the effect of transmembrane pressure (TMP) and cross-flow velocity (CFV) on permeate flux during the recovery and separation of glucose from cellulose hydrolysates by using membrane reactor was investigated. Two-stage pretreatment will be performed by using dilute sodium hydroxide (NaOH) and follow by dilute sulfuric acid (H_2SO_4) for about 24 hours at 75ºC respectively. Then, continued with enzymatic hydrolysis of cellulose with cellulase and cellobiase for 48 hours at 50 ºC and 150 rpm. Separation of glucose from cellulose hydrolysate will be performed by using ultrafiltration membrane for 60 minutes at 50ºC respectively. Then filtration method using ultrafiltration membrane was employed as a function of transmembrane pressure (TMP) and cross flow velocity (CFV), in order to identify their effects on the membrane flux and subsequently determine its optimum condition using response surface methodology (RSM). Filtration process was conducted at five different values of TMP and CFV range from 1 to 3 bars and 0.06 to 0.22 m/s respectively. The membrane flux after optimization was 116.655 $L/m^2.h$. The optimum conditions at TMP and CFV were found at 1 bar and 0.18 m/s.

ABSTRAK

Habuk kayu daripada kayu keras mengandungi sejumlah besar selulosa dan hemiselulosa. Hidrolisis enzim selulosa mempunyai potensi yang lebih besar dalam memenuhi makanan global dan permintaan tenaga dengan menghasilkan gula penurun seperti glukosa. Dalam kajian ini, kesan tekanan transmembran (TMP) dan halaju aliran silang (CFV) terhadap fluks semasa pemulihan dan pengasingan glukosa daripada hidrolisat selulosa dengan menggunakan reaktor membran telah dikaji. Dua peringkat pra-rawatan telah dilakukan dengan menggunakan natrium hidroksida cair (NaOH) dan diikuti dengan pra-rawatan menggunakan asid sulfurik (H_2SO_4) selama 24 jam pada suhu 75ºC. Kemudian, selulosa dihidrolisis oleh enzim selulase dan selubiase selama 48 jam pada 50ºC dan 150 rpm. Pengasingan glukosa daripada hidrolisat selulosa dilakukan menggunakan penapis ultra membran selama 60 minit pada 50ºC. Kesan tekanan transmembran (TMP) dan halaju aliran silang (CFV) terhadap fluks dikaji dan ditentukan keadaan optimumnya menggunakan kaedah tindakbalas permukaan (RSM). Proses penapisan telah dijalankan pada lima nilai TMP dan CFV yang berbeza dalam linkungan 1 hingga 3 bar dan 0.06 hingga 0.22 m/s. Nilai fluks maksimum terhasil adalah sebanyak 116.655 $L/m^2.h$. Keadaan optimum bagi kesan tekanan transmembran (TMP) dan halaju aliran silang (CFV) adalah pada 1 bar dan 0.18 m/s.

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LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Degree C
C	Carbon
cm	Centimetre
g	Gram
J	Flux
kDa	Kilo Dalton
kg	Kilogram
L	Liter
$L/m^2.h$	Liter per meter square per hour
m	Meter
M	Molarity
ml	Millilitre
%	Percentage

LIST OF ABBREVIATIONS

Adj R^2	Adjusted R^2
ANOVA	Analysis of variance
CCD	Central composite design
CFV	Cross flow velocity
DNS	Dinitrosalicylic
DOE	Design of experiment
Et al	An others
FTIR	Fourier Transform Infrared
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
NaOH	Sodium hydroxide
OFAT	One factor at a time
pH	Potentiometric hydrogen ion concentration
Q	Permeate flow rate in liter per minute
rpm	Rotary per minute
RSM	Response surface methodology
SEM	Scanning electron microscopy
TMP	Transmembrane pressure
MWCO	Molecular weight cut off

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Logging activities in Malaysia was one of important economic activity, however the sodas generated from processing the wood was wasted. The sawdust could be used to extract reducing sugar by using membrane reactor. Enzymatic hydrolysis of cellulose to produce reducing sugar such as glucose has larger potential in fulfill global food and energy demand. Cellobiose obtained by enzymatic hydrolysis of cellulose and cellulose rich materials such as sawdust (meranti). Glucose is separated by membrane reactor from enzyme cellulase and cellobiase.

Lignocelluloses biomass primarily consists of cellulose, hemicelluloses, and lignin which are usually being used as raw materials in the production of ethanol. Lignocelluloses biomass is believed to be less expensive and more plentiful than either starch or sucrose containing feedstock. Forest residues such as sawdust and wood bark are believed to be one of the most abundant sources of sugars, although much research has been reported on herbaceous grass such as switch grass, agricultural residue such as corn stover and municipal waste (Hu *et al*., 2008).

Besides that, the polysaccharides namely; cellulose and hemicelluloses present in the lignocelluloses biomass need to be hydrolyzed with acids or enzymes in order to produce fermentable sugars. Pretreatment is an important tool for practical cellulose conversion processes. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. Several studies have shown the potential of sodium hydroxide pretreatment on a variety of lignocellulosic materials. Sodium hydroxide pretreatment can enhances lignocelluloses digestibility by increasing internal surface area, decreasing the degree of polymerization and the crystallinity of celluloses, and separating structural linkages between lignin and carbohydrates effectively which will decreased lignin content (Wang *et al.*, 2010).Apart from that, dilute acid pretreatment has been widely investigated due to its effectiveness and inexpensive method of pretreatment compared to other pretreatment methods. The dilute sulfuric acid pretreatment can effectively solubilized hemicelluloses into monomeric sugars and soluble oligomers, thus improving cellulose conversion (Sun and Cheng, 2005). Thus, the combination of this two pretreatment to recovery of celluloses from different biomasses especially from wood will be one of the most interesting industrial processes in the near future.

In biotechnology industries, membrane application is gradually emerge as a powerful bioseparation for purification, fractionation, separation and concentration of bioproducts (Sakinah *et al.*, 2008). Pressure driven membrane filtration, one of membrane separation processes has been used to separate and concentrate the hemicelluloses extracted from wood (Mohammad, 2008). This procedure could be used for the cellulose separation. Membrane processes are generally classified into different categories which ranging from reverse osmosis and nanofiltration to ultrafiltration and cross-flow microfiltration that could be used to separate the cellulose.

1.2 PROBLEM STATEMENT

Membrane fouling is one of the problems that limit the use of membrane separation due to slowdown the reaction and lead to filtration resistance. Thus, decrease the efficiency to separate glucose from cellulose hydrolysates. Membrane filter cannot be recycle and need to change because of fouling problems. Hence it will increase the operational cost. Other than that, enzyme used in enzymatic reaction cannot be recycled if the reaction process did not take place in membrane reactor.

1.3 OBJECTIVES

The objective of this study is to retain enzyme cellulase and separate glucose from cellulose hydrolysate using membrane reactor. The study will be specified on the effect of transmembrane pressure and cross flow velocity based on:

- Effect of transmembrane pressure for glucose separation from reaction mixture.
- Effect of cross flow velocity for glucose separation from reaction mixture.
- To optimize and determine optimum condition for separation of glucose from reaction mixture.

1.4 SCOPE OF STUDY

In order to achieve the objective of the research, the optimum operating parameters; and flux during separation process will be observed. The optimum operating conditions are transmembrane pressure (TMP) and cross flow velocity (CFV) is important to obtain the high flux of cellulose recovery with less possibility of membrane fouling. The separation process is performing at transmembrane pressure varied from 0.5 to 2 bars while the cross-flow velocity is varied from 1.2 to 4 m/s (Carrere et al., 1998). The amount of glucose that has been filtered can be determined by using Dinitrosalicylic Colorimetric Method (DNS**)**. The optimization of glucose separation can be done by the Response Surface Methodology (RSM). This method decrease the period of research instead of maximizes the response.

1.5 RATIONALE AND SIGNIFICANCE OF STUDY

Raw material used can be considered as a low cost because sawdust was abundant and inexpensive in Malaysia. The composition of the cellulose is plenty in sawdust. The reuse of sawdust can also reduce the environmental pollution. Besides that, the production of cellulose has a potential in a future because from the cellulose, many valuable product can be produce such as bio-ethanol. Membrane separation was chosen because it has been widely used and has successfully proven its efficiency in various type of industry. However, there was lacking of membrane reactor used for separation of glucose from cellulose hydrolysates. Membrane reactor is the best method to separate glucose from cellulose hydrolysate as the enzyme cellulase and cellobiase will be neglected back to the reactor.

CHAPTER 2

LITERATURE REVIEW

2.1 RAW MATERIAL

Cellulose, like starch, is a polymer of glucose. However, unlike starch, the specific structure of cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures that is water insoluble and resistant to depolymerization. The other carbohydrate component in lignocellulosics is hemicellulose, which, dependent on the species, is a branched polymer of glucose or xylose, substituted with arabinose, xylose, galactose, fucose, mannose, glucose, or glucuronic acid (Mosier et al., 2005). Pretreatment is an important tool for practical cellulose conversion processes, and is the subject of this article. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier et al., 2005).

Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (Jorgensen et al., 2007). The composition of these constituents can vary from one plant species to another. For example, hardwood has greater amounts of cellulose, whereas wheat straw and leaves have more hemicellulose (Sun and Cheng, 2002). Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack (Perez et al., 2002). Figure 2.1 shows the role of pretreatment in conversion of biomass.

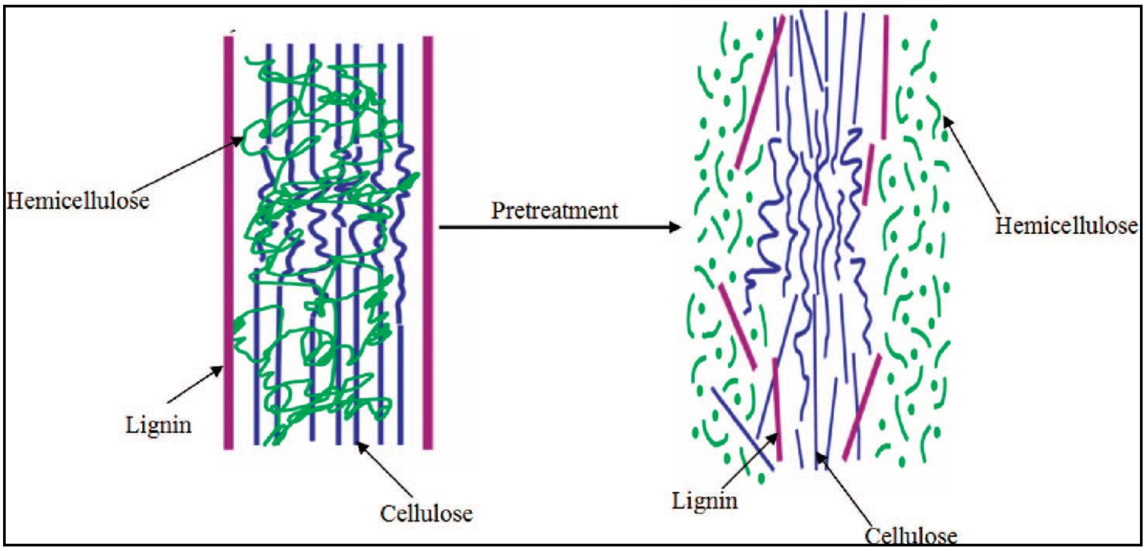

Figure 2.1: Schematic of the role of pretreatment in conversion of biomass

Source: Kumar et al., (2009)

In general, prospective lignocellulosic materials for fuel ethanol production can be divided into six main groups namely crop residues such as sugarcane bagasse, corn stover, wheat straw, rice straw, rice husks, barley straw, sweet sorghum bagasse, olive stones and pulp, hardwood such as aspen and poplar, softwood such as pine and spruce, cellulose wastes such as newsprint, waste office paper and recycled paper sludge, herbaceous biomass such as alfalfa hay, switch grass, reed canary grass, coastal Bermuda grass and timothy grass. Lignocellulosic biomass typically contains 55–75% carbohydrates by dry weight (Mosier et al., 2005). The carbohydrate content consists of mainly three different types of polymers, namely cellulose, hemicelluloses and lignin, which are associated which each other (Hendriks and Zeeman, 2009). Table 2.1 shows the general composition of selective lignocellulosic biomass containing cellulose, hemicelluloses and lignin.

Table 2.1: Cellulose, Hemicelluloses and Lignin contents in lignocellulosic biomass

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste papers from chemical pulps	60-70	10-20	5-10
Primary wastewater solids	8-15		2.7-5.7
Solid cattle manure	1.6-4.7	1.4-3.3	
Coastal Bermudagrass	25	35.7	6.4
Switch grass	45	31.4	12
Swine waste	6	28	N/A

Source: Kumar et al., (2009)

2.2 PRETREATMENT AND RECOVERY OF LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is mainly composed of cellulose, hemicelluloses and lignin. Cellulose was hydrolyzed to its monomeric constituents during enzymatic hydrolysis and then fermented to ethanol or other products. The cellulose biodegradation by cellulolytic enzymes is slow because of the networks between lignin-hemicelluloses were embedded the cellulose fibers. Therefore, pretreatment process is important to remove lignin and hemicelluloses, reduce cellulose crystallinity, and increase the porosity of the materials (Sun and Cheng, 2002) so that the produced cellulose is suitable for enzymatic hydrolysis. Pretreatment is required to disrupt the structure of lignocellulosic materials during cellulosic ethanol production, because the extensive interactions among cellulose, hemicelluloses and lignin, and the barrier nature of lignin minimize enzyme access to the carbohydrates and result in poor yields of fermentable sugars.

In general, pretreatment methods can be roughly divided into different categories such as physical pretreatment, physicochemical pretreatment, chemical pretreatment, biological, electrical, or a combination of these. The following pretreatment technologies have promise for cost-effective pretreatment of lignocellulosic biomass for biological conversion to fuels and chemicals (Kumar et al., 2009). Some pretreatment combines any two or all of these pretreatment and can be produce subcategories. Biological pretreatment has not attach much attention probably because of kinetic and economic considerations although there have been various research showing biological pretreatment can be an effective way to recover sugars from different species of biomass.

Physical and chemical pretreatments have been the subject of intensive research. Steam and water are usually excluded from being considered as chemical agent for pretreatment, since no extra chemical are added to the biomass. Physical pretreatment include comminuting, in which the particle sizes of the biomass are reduced with mechanical forces, steam explosion, and hydrothermalysis. Acids or bases promote hydrolysis and improve sugar recovery yield from cellulose by removing hemicelluloses and lignin during pretreatment. Sulfuric acid and sodium hydroxide are the most commonly used acid and base, respectively. Another approach for pretreatment is to use liquid formulations capable for acting as solvent for cellulose. Works with cellulose solvent systems have shown the enzymatic hydrolysis could be greatly improved, but the works mainly have been restricted to agricultural residues and herbaceous grass.

One of the main problems during the pretreatment and hydrolysis of biomass is the variability in the content of lignin and hemicelluloses. This variability depends on factors as the type of plant from which the biomasses obtained, crop age, method of harvesting, etc. this makes that no one of the pretreatment methods could be applied in a generic way for many different feed stocks. The future trends for improving the pretreatment of lignocellulosic feed stocks also include the production of genetically modified plant materials with higher carbohydrate content or modified plant structure to facilitate pretreatment in milder conditions or using hemicellulases. Several studies have shown the potential of sodium hydroxide pretreatment on a variety of lignocellulosic materials.

Furthermore, sodium hydroxide can enhances lignocelluloses digestibility by increasing internal surface area, decreasing the degree of polymerization and the crystallinity of celluloses, and separating structural linkages between lignin and carbohydrates effectively. Besides that, the digestibility of sodium hydroxide treated hardwood increased with the decrease of lignin content (Wang et al., 2010). Otherwise, the porosity of the lignocellulosic materials increases with the removal of the cross links which is lignin (Sun and Cheng, 2002). The major effect of alkaline pretreatments is the delignification of lignocellulosic biomass, thus enhancing the reactivity of the remaining carbohydrates (Wang et al., 2010). Besides that, based on the prominently researched and promising technology, dilute acid pretreatment was chosen as the method for treatment. The function of acid in this pretreatment is to break down the hemicelluloses and opens the remaining structure for subsequent enzymatic hydrolysis. Furthermore, reaction conditions which favor the production of xylose monomer while minimizing degradation to furfural is preferred so as they do not inhibit subsequent enzymatic hydrolysis.

Pretreatment of biomass with dilute sulfuric acid at high temperatures can effectively dissolve the hemicelluloses and increase the enzymatic digestibility of celluloses. Besides that, the pretreatment can be performed at the moderate temperature. These two conditions give different xylose yield as well as the glucose yield. However, the dilute acid pretreatment still give significant results based on the production of xylose and glucose. The reaction time can be extended to obtained higher yield of sugar with a period from days to week. The advantages of the dilute sulfuric acid were high reaction rates, low acid consumption, and low cost of sulfuric acid. Dilute sulfuric acid pretreatment is deserving attention due to relatively inexpensive and to produce high hemicelluloses recoveries and cellulose digestibilities (Lee et al., 2009). Therefore it has been assayed on a variety of substrates.

The application of dilute acid pretreatment to woody biomass can achieve some level of success so that can provide satisfactory cellulose conversion with certain hardwood species (Zhu and Pan, 2009). The dilute sulfuric acid pretreatment can effectively solubilized hemicelluloses into monomeric sugars, thus improving cellulose conversion. Compared to other pretreatment methods, it is especially useful for the conversion of xylan in hemicelluloses to xylose that can be further fermented to ethanol by many microorganisms (Sun and Cheng, 2005). Otherwise, dilute sulfuric acid pretreatment is effective because it is relatively inexpensive and due to high hemicelluloses recovery and cellulose digestibility (Cara et al., 2008). Besides that, dilute acid pretreatment with sulfuric acid has been extensively researched because it is inexpensive and effective, although other acid such as nitric acid, hydrochloric acid and phosphoric acid has also been tested (Hu et al., 2008).

2.3 GLUCOSE (PRODUCT)

Glucose is by far the most common carbohydrate and classified as a monosaccharide, an aldose, a hexose, and is a reducing sugar. It is also known as dextrose, because it is dextrorotatory; meaning that as an optical isomer is rotates plane polarized light to the right and also an origin for the D designation.

Glucose can be thought of as a derivative of hexane (a 6-carbon chain) with -OH groups attached to every carbon except the endmost one, which exists as an aldehyde carbonyl. However because the chain is flexible it can wrap around until the 2 ends react together to form a ring structure. Thus a solution of glucose can be thought of as a rapidly changing mixture of rings and chains, continually inter converting between the 2 forms.

In reality, an aqueous sugar solution contains only 0.02% of the glucose in the chain form, the majority of the structure is in the cyclic chair form. Since carbohydrates contain both alcohol and aldehyde or ketone functional groups, the straight-chain form is easily converted into the chain for, hemiacetal ring structure. Due to the tetrahedral geometry of carbons that ultimately make a 6 member stable ring, the OH on carbon 5 is converted into the ether linkage to close the ring with carbon 1. This makes a 6 member ring which had five carbons and one oxygen.

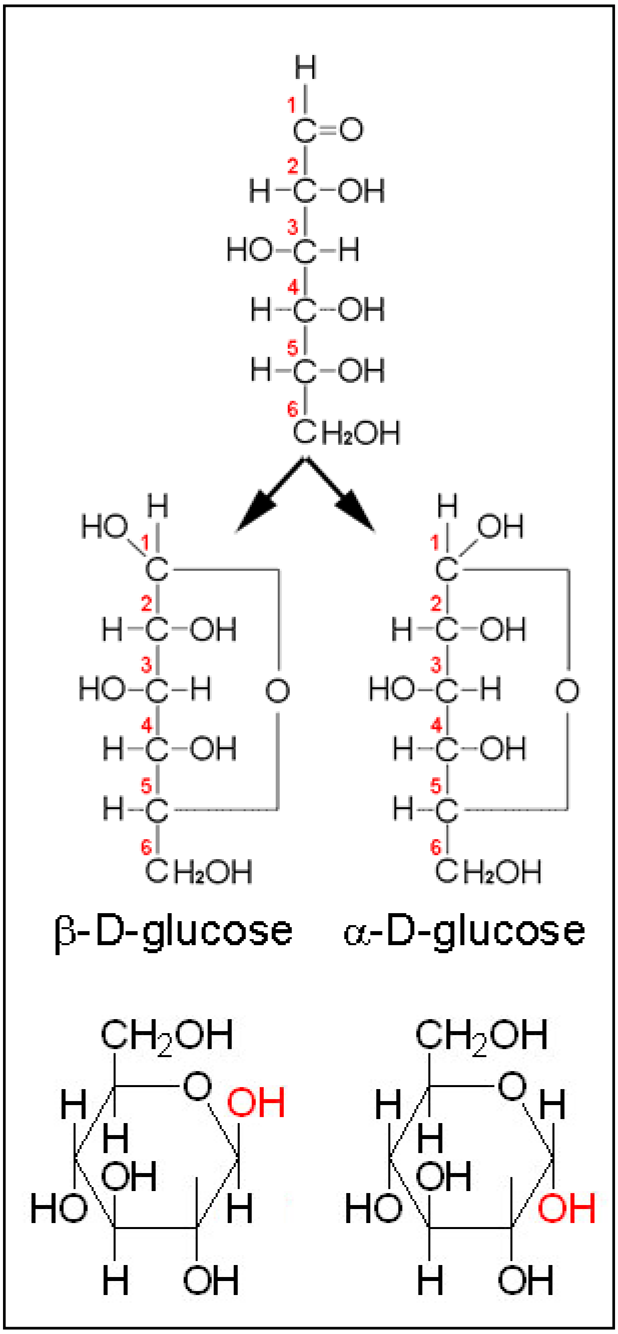

Figure 2.3: Structure of glucose

Source: http://alevelnotes.com/Monosaccharides/64

2.4 SEPARATION OF LIGNOCELLULOSIC BIOMASS RECOVERY

Separation processes such as sedimentation, filtration, membrane separation and centrifugal separations can be used for fractionating the wood extract. For the success of any molecular or ionic separation process downstream from wood hydrolysis and extraction, the extracts must be relatively clean and particles free (Duarte et al., 2010). This is particularly important since fouling and flux decay in nanofiltration or reverse osmosis applications can render these separations unviable on large scale.

Research has been conducted by Alriols et al., (2010) on the combined organosolv ethanol pretreatment with membrane ultrafiltration technology to treat the non-woody biomass feedstock of the species *miscanthus sinensis*. The lignin fraction with specific molecular weight was obtained by membrane ultrafiltration as it proportioned excellent fractionation capability with low chemicals consumption and low energy requirements. Besides that, acetic acid produced from the hydrolysis of herbaceous biomass such as corn stover was conventionally being separate and removed by chromatography method using resin column. Due to certain limitation, adsorptive microporous membrane has been used to remove acetic acid from corn stover hydrolysates (Wickramasinghe et al., 2008).

Furthermore, the separation of hemicelluloses from wood hydrolysates has been reported (Mohammad, 2008). The retention of hemicelluloses using two filtration steps was found to almost complete where the fouling ability of the used membrane was relatively low. The flux obtained at the first filtration was 165 $kg/m^2.h$ at 1 bar with 18% of membrane fouling and 24 $kg/m^2.h$ of flux at 10 bars with 30% of membrane fouling at second filtration.

2.5 MEMBRANE PROCESS

Membrane processes are mass transfer unit operations utilized for separation process either liquid-liquid or gas-liquid mixtures. Membrane is an ultra thin semi permeable barrier separating two fluids and allows the transport of certain species through the barrier from one fluid to the other. It is this permeability that gives the membrane its utility and potential to separate a variety of process streams. The most universally employed membranes are composed of organic polymers. Otherwise, type of membrane from metal, ceramic, liquid and gas membranes are also used. In all membrane methods, the membrane separates the fluid passing through it into a permeate (that which passes through) and a retentate (that which is left behind). When the membrane is chosen so that it is more permeable to one constituent than the other, then permeate will be richer in the first constituent than the retentate (Kumar et al., 2010).

2.6 MEMBRANE SEPARATION OF GLUCOSE

Color removal from sugar syrup and the improvement of its sugar purity using ultrafiltration has great advantage. Membrane separation has been studied for color removal from green sugar syrup (Gyura et al., 2005). Ultrafiltration membranes with porosity ranging from 6 to 20 kilo Dalton (kDa) were used to remove color from raw 16 sugar cane solution. The permeate was decolorized by 58% using a 6 to 8 kDa membrane at a flux of 35.32 L/m^2.h, which gave the best results. The 15 to 20 kDa membrane only removed 50% of the color at a flux of 15.78 L/m^2.h.

Membrane separation has been performed as an alternative method for the recovery of xylitol from the fermentation broth of hemicelluloses hydrolysates because it has the potential for energy savings and higher purity (Affleck, 2000). A 10,000 nominal molecular weight cutoff (MWCO) polysulfone membrane was found to be the most effective for the separation and recovery of xylitol. The membrane allowed 82.2 to 90.3% of xylitol in the fermentation broth to pass through the membrane.

Otherwise, membrane filtration has also been used as an alternative for the separation and purification of hemicelluloses extracted from wood and annual crops (Mohammad, 2008). The outcome shows that the permeate flux through ultrafiltration and tight ultrafiltration membranes was relatively high. The fouling ability of the used membranes was relatively low. In addition, the retention of hemicelluloses using two filtration steps was almost complete.